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ATS Labs Project # A14868
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ATS LABS

PROTOCOL

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Bovine Viral Diarrhea Virus as a Surrogate Virus for Human Hepatitis C Virus

PROTOCOL NUMBER

HSP01021413.BVD

PREPARED FOR

HSP USA LLC
3111 Route 38, Suite 11, #310
Mount Laurel, NJ 08054

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PREPARED BY

Mary J. Miller, M.T.
Senior Virologist

DATE

February 14, 2013

EXACT COPY
INITIALS JC DATE 5/3/13

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Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Bovine Viral Diarrhea Virus as a Surrogate Virus for Human Hepatitis C Virus

SPONSOR: HSP USA LLC
3111 Route 38, Suite 11, #310
Mount Laurel, NJ 08054

TEST FACILITY: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE

The purpose of this study is to evaluate the virucidal efficacy of a test substance against Bovine Viral Diarrhea virus to be used as a surrogate virus for Human Hepatitis C virus for registration of a product as a virucide. The test procedure is to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to, one or more of the following agencies as indicated by the Sponsor: U.S. Environmental Protection Agency (EPA), Health Canada Therapeutic Products Directorate (TPD) and Australian Therapeutic Goods Administration (TGA).

Human Hepatitis C virus (HCV), a member of the Flaviviridae Family of enveloped RNA-containing viruses, presents a serious public safety concern. However, at present, there is no reliable *in vitro* infectivity assay for this virus. Bovine Viral Diarrhea virus, also a member of Flaviviridae Family, serves as a valuable model virus for human Hepatitis C virus, since these viruses share many similar characteristics.

TEST SUBSTANCE CHARACTERIZATION

Test substance characterization as to content, stability, solubility, storage, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor. The test substance shall be characterized before the experimental start date of this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to ATS Labs.

SCHEDULING AND DISCLAIMER OF WARRANTY

Experimental start dates are generally scheduled on a first-come/first-serve basis once ATS Labs receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is March 5, 2013. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of March 29, 2013. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

If a test must be repeated, or a portion of it, because of failure by ATS Labs to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of ATS Labs nor any of its employees are to be used in advertising or other promotion without written consent from ATS Labs.

The Sponsor is responsible for any rejection of the final report by regulatory agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the ATS Labs final report and notify ATS Labs of any perceived deficiencies in these areas before submission of the report to the regulatory agency. ATS Labs will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Regulatory agencies require that a specific virucidal claim for a disinfectant intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed virus. The agency will accept adequate data generated by any appropriate technique in support of a virucidal efficacy claim. This is accomplished by treating the target virus with the disinfectant (test substance) under conditions, which simulate as closely as possible, in the laboratory, the actual conditions under which the disinfectant is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting virological data. The bovine turbinate cell line, which supports the growth of the Bovine Viral Diarrhea virus, will be used in this study. The experimental design in this protocol meets these requirements.

TEST PRINCIPLE

A film of virus, dried on a glass surface, is exposed to the test substance for a specified contact time. At the end of the exposure time, the virucidal and cytotoxic activities are removed from the virus-test substance mixture, and the mixture is assayed for viral infectivity by an accepted assay method. Appropriate virus, test substance cytotoxicity, and neutralization controls are run concurrently.

STUDY DESIGN

Dried virus films will be prepared in parallel and used as follows:

The appropriate number of films for each batch of test substance assayed per exposure time requested.

The appropriate number of films for virus control titration (titer of virus after drying) per exposure time requested.

At the end of the specified exposure time, resuspended virus-test substance mixtures will be detoxified and made non-virucidal by immediately adding the contents to a Sephadex gel filtration column followed by 10-fold serial dilutions in test medium. Each dilution is assayed for viral infectivity by inoculation into bovine turbinate cell cultures. The resuspended virus control films and each batch of test substance alone (for toxicity determinations) will be treated in exactly the same manner. For analysis of infectivity, the cell cultures will be held for the appropriate incubation period and microscopically observed for the presence of the test virus or cytotoxicity. For analysis of cytotoxicity, the viability of cultures inoculated with dilutions of each test and cytotoxicity control will be determined. Uninfected indicator cell cultures will be maintained to serve as a negative control. In addition to the above titrations for infectivity and toxicity, the residual virucidal activity of the test substance following neutralization will be determined by adding a low titer of stock virus to each dilution of the detoxified test substance (toxicity control dilutions). The resulting mixtures of dilutions are assayed for infectivity in order to determine the dilution(s) of test substance at which virucidal activity, if any, is retained.

The following table outlines the specific parameters that will be tested:

PARAMETERS TESTED FOR VIRUCIDAL EFFICACY ASSAY		
Test or Control Group	Dilutions Assayed Per Carrier (log_{10})	Culture Wells per Dilution
Negative Controls	N/A	2-4
Input Virus Control (not dried)	-4,-5,-6,-7*	4
Dried Virus Control ▲	-4,-5,-6,-7*	4
Test Samples (per batch of test substance) ▲	-1,-2,-3,-4	4
Cytotoxicity Control (per batch of test substance)	-1,-2,-3	2
Neutralization Control (per batch of test substance)	-1,-2,-3	2

* The ending dilution assayed may change depending on the titer of the virus.
▲ Performed in duplicate for data submitted to U.S. EPA and/or Australia TGA regulatory agencies. Five replicates assayed for data submitted to Health Canada TPD.

VIRUS

The Oregon C24v – genotype 1 strain of Bovine Viral Diarrhea virus (BVDV) received from the National Veterinary Services Laboratories (NVSL), Ames, IA, will be used for this study. Alternatively, the NADL strain of Bovine Viral Diarrhea virus obtained from the American Type Culture Collection (VR-1422) may be used. The strain and source of the virus will be documented in the raw data and final report. Stock virus is prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells are disrupted and cell debris removed by centrifugation. The supernatant is removed, aliquoted, and the high titer stock virus may be stored at ≤-70°C until the day of use. On the day of use, the appropriate number of aliquots are removed, thawed, combined (if applicable) and maintained at a refrigerated temperature until used in the assay. **Note:** If the Sponsor requests an organic soil load challenge, horse serum will be incorporated into the stock virus aliquot. The percent horse serum contained in the stock virus aliquot will be adjusted to yield the percent soil load requested. If an alternate organic soil load is requested, the stock virus aliquot will be adjusted to yield the percent organic soil load requested.

INDICATOR CELL CULTURES

Cultures of bovine turbinate (BT) cells were originally obtained from the American Type Culture Collection (ATCC CRL-1390). The cells are propagated at ATS Labs, and the cultures are maintained and used at the appropriate density in tissue culture labware at 36-38°C in a humidified atmosphere of 5-7% CO_2. BT cells obtained from an alternate, reputable source may be used. The source of the cells will be specified in the raw data and final report.

This cell line has historically been used as the cell line for propagation and detection of BVDV. The cultures are commercially available, can be serially propagated, and are capable of showing cytopathic effect in the presence of the virus. The use of BT cells is a standard industry practice for propagation and detection of BVDV as indicated by the American Type Culture Collection (ATCC) recommendation of using BT cells for the growth of Bovine Viral Diarrhea virus. The virus is also capable of propagation in this cell line to titers suitable for efficacy testing.

All cell culture documentation is retained for the cell cultures used in this assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

TEST MEDIUM

The test medium used for this assay is Minimum Essential Medium (MEM) supplemented with 1-10% (v/v) non-heat inactivated horse serum. The medium may also be supplemented with one or more of the following: 10 µg/mL gentamicin, 100 units/mL penicillin and 2.5 µg/mL amphotericin B. The composition of the test medium may be adapted. The composition of the medium will be specified in the final report.

PREPARATION OF THE TEST SUBSTANCE

The dilution of the test substance used will be as recommended by the Sponsor. The test substance will be pre-equilibrated to the desired test temperature if applicable. The number of batches of test substance assayed will be as requested by the Sponsor.

PREPARATION OF THE VIRUS FILMS

Films of virus will be prepared by spreading 200 µL of virus inoculum uniformly over the bottoms of the appropriate number of 100 X 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus will be air-dried at 10°C-30°C until visibly dry (≥20 minutes). The drying conditions (temperature and humidity) will be appropriate for the test virus for the purpose of obtaining maximum survival following drying. The actual drying conditions, drying time and calibrated timer used will be clearly documented.

For U. S. EPA, Australian TGA, and internal/other use only, two dried virus film per batch of test substance will be assayed unless otherwise requested. For Health Canada TPD, five dried virus films per batch of test substance will be assayed unless otherwise requested. If multiple regulatory agencies are chosen, the greater number of virus films will be assayed.

TEST METHOD

Preparation of Sephadex Gel Filtration Columns

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus is separated from the test substance by filtration through Sephadex gel. The type of Sephadex used will be specified in the final report. On the day of testing, Sephadex columns are prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns are now ready to be used in the assay.

Input Virus Control

On the day of testing, the stock virus utilized in the assay will be titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

Treatment of Virus Films with the Test Substance

For each batch of test substance assayed, the appropriate number of dried virus films are individually exposed to a 2.0 mL aliquot of the use dilution of the test substance (liquid products), or to the amount of spray released under use conditions (spray products) and held covered for the specified exposure time and temperature. A calibrated timer will be used for timing the exposure. The actual temperature will be recorded. Just prior to the end of the exposure time, the plates are individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrate (10^{-1} dilution) is then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. To further aid in the removing of the cytotoxic effects of the test substance to the indicator cell cultures, individual dilutions may be passed through additional individual Sephadex columns.

Treatment of Dried Virus Control Films

The appropriate number of virus films are prepared as described previously. The virus control films are run in parallel to the test virus but a 2.0 mL aliquot of test medium is added in lieu of the test substance. The virus control films are held covered and exposed to the test medium for the same exposure time and at the same exposure temperature as the test films are exposed to the test substance. A calibrated timer will be used for timing the exposure. The actual temperature will be recorded. Just prior to the end of the exposure time, the virus control films are individually scraped as previously described and at the end of the exposure time the mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger. The filtrate (10^{-1} dilution) is then titered by 10-fold serial dilution and each dilution is then assayed for infectivity. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the virus control will be passed through additional individual Sephadex columns. The purpose of this control is to determine the titer of the dried virus that was exposed to the test system. The titers of the dried virus control replicates will be used to calculate the log reduction or MPN following the exposure of the virus to the test substance.

Cytotoxicity Control
A 2.0 mL aliquot of each batch of test substance (liquid products) or the amount of the test substance recovered when sprayed onto a sterile petri dish (spray products), is filtered through a Sephadex column utilizing the syringe plunger The filtrate is then titered by 10-fold serial dilution in test medium and inoculated into cell cultures and assayed for cytotoxicity. For spray products, the cytotoxicity control will be held covered for the longest requested exposure time at the requested exposure temperature. A calibrated timer will be used for timing the exposure. If additional Sephadex columns were used to further reduce the cytotoxic effects of the test substance to the indicator cell cultures in the test substance assay, the same dilutions of the cytotoxicity control will be passed through additional individual Sephadex columns. The cytotoxicity of the cell cultures is scored at the same time as the virus-test substance and virus control cultures.

When the organic soil load contains blood, a dried film comprised of test medium with the requested concentration of blood may be prepared for each batch of test substance assayed. The prepared film(s) will be held for the longest requested exposure time and temperature and will be treated as previously described. The same procedure may be utilized for other organic soil load components as needed.

Assay of Non-Virucidal Level of Test Substance (Neutralization Control)
Each dilution of the neutralized test substance (cytotoxicity control dilutions) will be challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, is retained. Dilutions that show virucidal activity will not be considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures will be inoculated with a 100 µL aliquot of each dilution in duplicate. A 100 µL aliquot of low titer stock virus will be inoculated into each cell culture well and the indicator cell cultures will be incubated along with the test and virus control plates.

Infectivity Assay
The BT cell line, which exhibits cytopathic effect (CPE) in the presence of Bovine Viral Diarrhea virus, will be used as the indicator cell line in the infectivity assays. Cells contained in multiwell culture dishes will be inoculated in quadruplicate with a 100 µL aliquot of the dilutions prepared from the test and control groups. The cytotoxicity and neutralization control dilutions will be inoculated in duplicate. Uninfected indicator cell cultures (cell controls) will be inoculated with test medium alone. The cultures are incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cytopathic effect of the virus can be described as rounding, dark, with a granular appearance. The cultures will be microscopically scored periodically for approximately seven days for the absence or presence of CPE, cytotoxicity and for viability. All cultures (cells) will be microscopically examined and the results recorded.

The determination of CPE can be subjective, therefore to verify the CPE reading, on the final day of incubation a direct immunofluorescence assay (DFA) which is a more sensitive detection method to detect the virus in the host cells will be performed using a fluorescein conjugated antibody specific for BVDV, received from VMRD, Inc., Pullman, WA, or other suitable vendor. The DFA will be performed only on the first inoculated dilution of the test and a dilution of the dried virus control exhibiting CPE. Alternatively, more than one dilution of the dried virus control or test may be stained. A set of negative controls (cell controls) will be stained as well. If >50% of the monolayer of the first inoculated dilution is affected by test substance cytotoxicity, then the first dilution not showing test substance cytotoxicity will also be stained and read. The DFA is performed by first aspirating the medium from each cell culture well and rinsing the cells with an approximate 1.0 mL aliquot of phosphate buffered saline (PBS) or other suitable rinse agent. The PBS is aspirated and an approximate 1.0 mL of chilled ethanol is added to each well to fix the cells for a minimum of two hours at 2-8°C. The cells are rinsed twice with PBS or other suitable rinse agent. An approximate 0.2 mL aliquot of fluorescein conjugated antibody (diluted per vendor recommendation, if applicable) is added to each well. The cultures are incubated in a humidified atmosphere of 36-38°C for a minimum of 30 minutes. Following the incubation, the cells are rinsed twice with an approximate 1 mL aliquot of PBS or other suitable rinse agent. The PBS is removed; the plates are blotted to remove the excess PBS and then observed microscopically using ultraviolet light for infectivity. A cell which is positive for the test virus will fluoresce, cells negative for the test virus will not. The cell culture plates may be stored at 2-8°C wrapped in foil for up to one week prior to reading microscopically.

DATA ANALYSIS

Calculation of Titers

Viral and cytotoxicity titers will be expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity (TCD_{50}), respectively, as calculated by the method of Spearman Karber.

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Statistical Methods

The $\log_{10}$ reduction in infectivity will be calculated using a Most Probable Number (MPN) statistical method. The Statistical Analysis of Virucide Carrier Test Data Template for calculating the Log Reduction (LR) and Associated Standard Error (SE); version of 6 January, 2010, obtained from Martin A. Hamilton, Big Sky Statistical Analysts LLC, Bozeman, MT, USA, will be used.

Note: If the data will be submitted to Health Canada TPD ONLY, the following calculation for $\log_{10}$ reduction may be used instead of the MPN statistical method. The average titer of the replicates will be calculated and the average titer will be used to calculate the log reduction in viral titer of the individual test replicates.

Calculation of Log Reduction

Average Dried Virus Control Log $TCID_{50}$ – Test Substance Log $TCID_{50}$ = Log Reduction

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

The specialized virucidal testing section of ATS Labs maintains Standard Operating Procedures (SOPs) relative to virucidal efficacy testing studies. Virucidal efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including virus and cell stocks for purposes of identification, receipt and use of chemical reagents including cell culture medium components, etc. These procedures are designed to document each step of virucidal efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each virucidal efficacy test is assigned a unique Project Number when the Study Director initiates the protocol for the study. This number is used for identification of the test culture plates, etc. during the course of the test. Test culture plates are also labeled with reference to the test virus, experimental start date, and test product. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: N/A

STUDY ACCEPTANCE CRITERIA

Only the applicable acceptance criteria and references for the regulatory agency reviewing the data will be included in the final report.

U.S. EPA Submission
A valid test requires 1) that at least 4 $\log_{10}$ of infectivity be recovered from the dried virus control film; 2) that when cytotoxicity is evident, at least a 3-log reduction in titer is demonstrated beyond the cytotoxic level; 3) that the cell controls be negative for infectivity. If any of the previous requirements are not met, the test may be repeated under the current protocol number. **Note:** An efficacious product must demonstrate complete inactivation of the virus at all dilutions.

Health Canada TPD Submission
A valid test requires 1) at least a 4-log infectivity be recovered from the dried virus control film beyond the cytotoxic level of the test substance; 2) that the cell controls be negative for infectivity. If any of the previous requirements are not met, the test may be repeated under the current protocol number. **Note:** An efficacious product must demonstrate at least a 3 $\log_{10}$ reduction in viral titer beyond the cytotoxic level of the test substance.

Australian Therapeutic Goods Administration
A valid test requires 1) that at least 4 $\log_{10}$ of infectivity be recovered from the dried virus control film; 2) that when cytotoxicity is evident, at least a 3-log reduction in titer is demonstrated beyond the cytotoxic level; 3) that the cell controls be negative for infectivity. If any of the previous requirements are not met, the test may be repeated under the current protocol number. **Note:** An efficacious product must demonstrate complete inactivation of the virus at all dilutions.

FINAL REPORT

The report will include, but not be limited to, identification of the sample and date received, dates on which the test was initiated and completed, identification of the virus strain used and composition of the inoculum, description of cells, medium and reagents, description of the methods employed, tabulated results, calculated titers for infectivity and cytotoxicity, and a conclusion as it relates to the purpose of the test. .

PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

TEST SUBSTANCE RETENTION

Test substance retention shall be the responsibility of the Sponsor. Unused test substance will be discarded following study completion unless otherwise requested.

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RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at ATS Labs. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of the final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at ATS Labs. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study, which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Response, E 1053 (current version).
2. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Response, E 1482 (current version).
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Public Health Uses of Antimicrobial Agents, March 12, 2012.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces – Efficacy Data Recommendations, March 12, 2012.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Canadian General Standards Board, Minister of Public Works and Government Services, August 1997. Assessment of Efficacy of Antimicrobial Agents for Use on Environmental Surfaces and Medical Devices. CAN/CGSB-2.161-97.
8. Health Canada Therapeutic Products Directorate, October 29, 2007. Guidance Document: Disinfectant Drugs, Health Products and Food Branch.
9. Australian Therapeutic Goods Administration (TGA), February 1998. Guidelines for the Evaluation of Sterilants and Disinfectants.
10. Australian Therapeutic Goods Administration (TGA), February 1998. Therapeutic Goods Order No. 54: Standard for Disinfectants and Sterilants.
11. Australian Therapeutic Goods Administration (TGA), March 1997. Therapeutic Goods Order No. 54A: Amendment to Standard for Disinfectants and Sterilants (TGO 54).
12. Australian Therapeutic Goods Administration (TGA), July 2005. Draft Guidelines for the Evaluation of Household/Commercial and Hospital Grade Disinfectants.
13. Statistical Analysis of Virucide Carrier Test Data Template for calculating the Log Reduction (LR) & associated Standard Error (SE); version of 6 January, 2010. Martin A. Hamilton, Big Sky Statistical Analysts LLC, Bozeman, MT USA.

STUDY INFORMATION

(All sections must be completed prior to submitting protocol)

Test Substance (Name and Batch Number - exactly as it should appear on final report):
(HSP20) Lot 518, Lot 519 *

Expiration Date: 2 weeks after ~~expiration~~ receipt * Added per Sponsor, see email dated 4/1/13. JC 4/3/13

Product Description
- ☐ Quaternary ammonia
- ☐ Iodophor
- ☐ Peracetic acid
- ☐ Peroxide
- ☐ Sodium hypochlorite
- ☒ Other Hypochlorous acid

Test Substance Active Concentration (upon submission to ATS Labs): 140 ppm

Storage Conditions
- ☒ Room Temperature
- ☐ 2-8°C
- ☐ Other _____

Hazards
- ☒ None known: Use Standard Precautions
- ☐ Material Safety Data Sheet attached for each product
- ☐ As Follows: _____

Product Preparation
- ☒ No dilution required, Use as received (RTU)
- ☐ *Dilution(s) to be tested:

_____ defined as _____ + _____
(example: 1 oz/gallon) (amount of test substance) (amount of diluent)

- ☐ Deionized Water (Filter or Autoclave Sterilized)
- ☐ Tap Water (Filter or Autoclave Sterilized)
- ☐ AOAC Synthetic Hard Water: _____PPM
- ☐ Other _____

Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Virus: Bovine Viral Diarrhea virus as a surrogate virus for Human Hepatitis C virus

Exposure Time: 1 minute

Exposure Temperature:
- ☒ Room temperature (range to be based on specific regulatory agency requirements)
- ☐ Other: _____°C (please specify range)

Directions for application of aerosol/spray products (if applicable): _____
* JC 4/3/13
☑ Check here if spray instructions are not applicable.

Organic Soil Load
- ☐ 1% horse serum (minimum level that can be tested)
- ☒ 5% horse serum
- ☐ Other: _____

REGULATORY AGENCY(S) THAT MAY REVIEW DATA

- ☑ U.S. EPA
- ☐ Health Canada (Canadian TPD)
- ☑ Therapeutic Goods Administration (Australian TGA)
- ☐ Not applicable - For internal/other use only (Efficacy result will be based on U.S. EPA requirements)

TEST SUBSTANCE SHIPMENT STATUS

- ☐ Has been used in one or more previous studies at ATS Labs.
- ☐ Has been shipped to ATS Labs (but has not been used in a previous study).
  Date shipped to ATS Labs:_____ Sent via *overnight* delivery? ☐ Yes ☐ No
- ☑ Will be shipped to ATS Labs.
  Date of expected receipt at ATS Labs:_____
- ☐ Sender (if other than Sponsor):_____

COMPLIANCE

This study will be conducted in compliance with the EPA Good Laboratory Practices Regulations of 40 CFR Part 160 (Federal Register Notice [August 17, 1989]) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP Study)

PROTOCOL MODIFICATIONS

- ☑ Approved without modification
- ☐ Approved with modification

_____

_____

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Mr. Henry Dao TITLE: President/CEO

SIGNATURE: [signature] DATE: 3/27/13

PHONE: (856) 437 - 0688 FAX: (866) 799 - 8079 EMAIL: info@hsp-usa.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

ATS Labs:

NAME: Shaneen Conway
Study Director

SIGNATURE: Shaneen L. Conway DATE: 4/3/13
Study Director